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POSITIVE AND HOST-INDUCED NEGATIVE PHOTOTAXIS OF THE SYMBIOTIC WATER MITE *UNIONICOLA FORMOSA*

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Many animals respond to certain environmental parameters by an array of taxes and kineses. The initiation and maintenance of numerous symbiotic associations often involve such behavioral responses mediated by one or more sensory modality (Davenport, 1955, 1966). The symbiosis of the freshwater mite *Unionicola formosa* (Acarina: Unionicolidae) and the mussel *Anodonta imbecilis* (Schizodonta: Unionidae) includes several components potentially involving behavioral interactions between symbiont and host.

Female specimens of *U. formosa* deposit eggs in the gills of the host mussel. Larvae emerge in the spring, leaving the host and possibly becoming parasitic on chironomids (Jones, 1965; Böttger, 1976). They subsequently reinvade a mussel and embed in the gills, forming a quiescent transformational stage, the nymphochrysalis, from which emerges the sexually immature nymph. Whether nymphal *U. formosa* leave the host remains uncertain, but ultimately they enter host gills and form the quiescent teleiochrysalis from which the mature adult emerges. As many as 70 mature female mites may occupy the host mussel's mantle cavity, but rarely more than a single male (Roberts, 1977).

The first experimental analysis of a symbiosis involving unionicolid mites was that of Welsh (1930) who reported that *U. ypsilophora* was positively phototactic when washed free of any chemical influence of its host, *Anodonta cataracta*, but displayed negative phototaxis when tested in water containing host gill homogenate or in water from the mantle cavity of the mussel. Welsh (1930) contended that this negative phototaxis helped keep mites in the host's mantle cavity. Reversal of the mite's phototaxis was species-specific in that only tissue homogenate from the species of mussel with which mites had been associated in the field elicited the response (Welsh, 1931). Subsequent study (Welsh, 1932) revealed a positive photokinesis by *U. ypsilophora*, a behavioral response later employed by Waterman (1937) to determine the spectral sensitivity of this organism. To our knowledge no study to date has either characterized more fully the positive phototaxis of unionicolid mites or examined the host-induced negative phototaxis.

Preliminary experiments revealed that *U. formosa* is positively phototactic when tested in water which is free of any host influence and negatively phototactic in the presence of some chemical signal of host origin. This reversal of phototaxis occurs in males, females, nymphs and perhaps larvae. This paper

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examines the spectral and intensity sensitivity characteristics for both positive and host-induced negative phototaxis of the readily obtainable adult female mites. It is likely that the *U. ypsilophora* of Welsh (1930, 1931, 1932) and Waterman (1937) is in reality *U. formosa,* since the two forms are similar and *U. ypsilophora* apparently does not occur in North America (Mitchell, 1957).

Materials and Methods

Anodonta imbecilis with *Unionicola formosa* was collected from November 1976 to May 1977 from Par Pond, Aiken County, South Carolina. Adult female mites were removed from their hosts and held 1 to 10 days in artificial pond water (APW) (Prosser, 1973), which was changed daily and immediately before each series of experiments. Preliminary experiments revealed that phototactic behavior was unchanged within this time period. All animals were kept at 18 to 20° C under naturally occurring illumination from laboratory windows. Experiments were always performed between 1000 and 1600 hr.

Measurements of phototaxis were conducted in a clear lucite chamber (119 × 20 × 20 mm) which was provided with removable partitions that subdivided the chamber into five equal compartments. The chamber could be illuminated horizontally from one end with a Leitz slide projector (300 W tungsten filament bulb) to which was attached a filter holder. The light was filtered first through an infrared absorbing filter (Corning No. 1-75) and a "hot mirror" (Baird Atomic) to reduce the radiation above 700 nm. The light was then modified by the interposition of thin film filters (3-cavity; halfband pass 7 to 8 nm; Ditric Optics Co.) to control wavelength, and neutral density filters (Ditric Optics Co.) to control intensity. Light intensity was measured with a quantum sensor (Lambda Instrument Corporation Model LI-185).

The test medium in the chamber was either APW or APW to which had been added homogenate prepared as follows: mantle tissue was excised from healthy mussels and ground in cold APW in a tissue homogenizer, diluted with APW to 1.0 g wet weight tissue/10 ml APW, filtered through a 0.45 μ Millipore filter and frozen until used (no more than 5 days after preparation). Immediately prior to use the homogenate was thawed, refiltered, and diluted to a final concentration in the chamber of 0.02 g wet weight tissue/ml APW. Mantle tissue was selected for the homogenate, since it was never used by *U. formosa* as a site of egg deposition and only rarely for the transformational forms.

Mites were light-adapted for at least 1 hr under incandescent room lights plus a 60 W incandescent bulb or dark-adapted 1 hr or more. In tests using dark-adapted animals the mites were transferred to the test chamber under low intensity red light from a GE 25 W red incandescent bulb which was wrapped with several layers of red cellophane. All tests were performed at 20 to 22° C.

The experimental protocol consisted of placing 30 to 35 mites into the central compartment of the chamber to which the desired test medium had been added. Preliminary experiments revealed no significant interaction among mites at this density. The animals were held 30 sec in the dark following introduction into the test chamber. The partitions were then removed and the monochromatic test light turned on. Mites tested for positive phototaxis (no host homogenate pres-

ent) were stimulated for 60 sec, while those tested under conditions eliciting negative phototaxis (host homogenate present) were exposed to the light for 90 sec, since negative phototaxis took longer to be expressed. At the end of each test the partitions were reinserted and the number of mites in each section was recorded.

Two series of experiments were conducted. Spectral sensitivity was established for light- and dark-adapted mites by determining phototactic responsiveness upon stimulation with approximately equal quantal intensities of the test wavelengths. The different responsiveness of light- and dark-adapted mites during positive and negative phototaxis necessitated using several quantal intensities. Intensity sensitivity was examined by monitoring phototactic responsiveness to a range of intensities of 500 nm light.

The data are presented as percent response based on the number of mites in the section of the chamber closest to the light source (positive phototaxis) or the section farthest from the light source (negative phototaxis). Where appropriate, the data have been analyzed by the Kolmogorov-Smirnov goodness of fit test (Zar, 1974), which compared the distribution of mites in the five sections of the chamber under various test conditions. Control data were generated by repeating the experimental procedure without the stimulus light and monitoring the distribution of mites in the chamber.

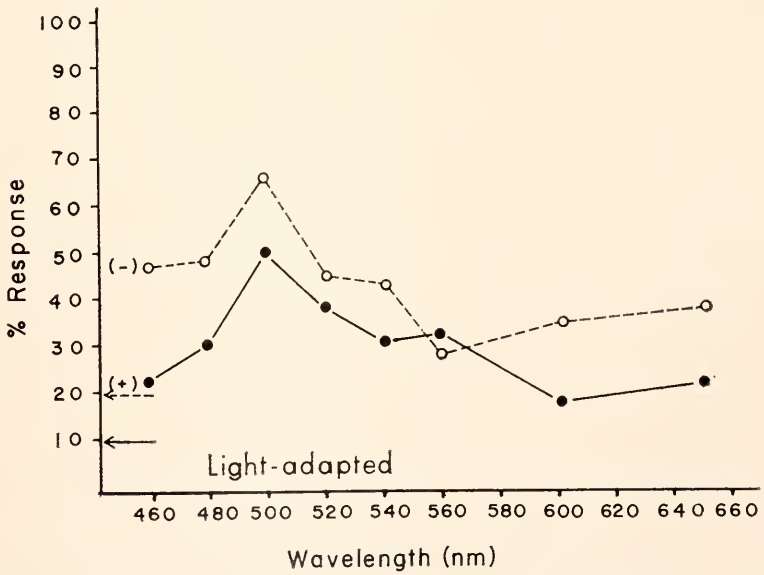

FIGURE 1. Spectral sensitivity curves of positive (solid line) and negative (dashed line) phototaxis of light-adapted *U. formosa*. Positive phototaxis was generated in clean artificial pond water (APW), and negative phototaxis was in APW plus host homogenate. Mean intensities (quanta/m^2/sec) for positive and negative phototaxis are $3.210 \times 10^{12} \pm 8\%$ and $2.248 \times 10^{15} \pm 10\%$, respectively. Arrows indicate the control levels. The N for each condition ranged from 62 to 69.

Results

Spectral sensitivity curves for positive and negative phototaxis of light-adapted female *U. formosa* are depicted in Figure 1. The magnitide of both the positive and negative responses was influenced by intensity (as revealed by other experiments), but the patterns expressed all were similar to those of Figure 1, with maximal sensitivity for both positive and negative phototaxis occurring around 500 nm. The positive phototaxis to 500 and 520 nm (Fig. 1) was significantly greater ($P < 0.05$, Kolmogorov-Smirnov) than that of controls or that associated with any other wavelength. Negative phototaxis was significantly greater ($P < 0.05$) at 500 nm than at any other wavelength.

Spectral sensitivity curves for dark-adapted animals are presented in Figure 2. Positive phototaxis had a less well-defined maximum for dark adapted mites and continued into longer wavelengths than did the positive response of light-adapted animals (Fig. 1). For example, positive respones at 500 to 560 nm were not different from each other, but were significantly different from controls and from responses at other wavelengths. Dark-adapted mites were maximally negatively phototactic at wavelengths of 460 to 500 nm. The response at 480 nm was significantly higher ($P < 0.05$) than at any other wavelength except 460 nm. Dark-adaptation also decreased the sensitivity of negatively phototactic mites to wavelengths longer than 500 nm. It is unclear why the patterns of positive and negative phototaxis agree for light-adapted animals (Fig. 1), but are dissimilar for dark-adapted mites (Fig. 2). However, the responses of dark-adapted

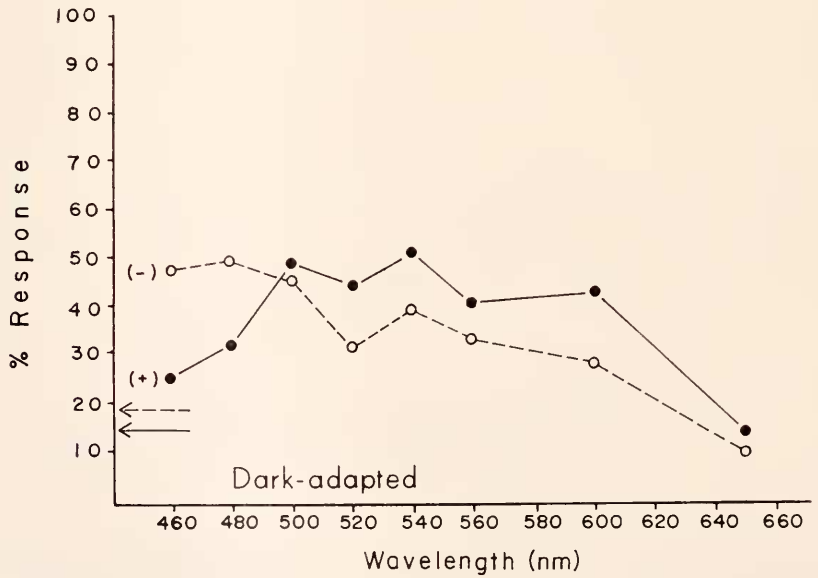

Figure 2. Spectral sensitivity curves of positive (solid line) and negative (dashed line) phototaxis of dark-adapted mites. Positive phototaxis was generated in APW, while negative phototaxis was in APW plus host homogenate. Mean intensities (quanta/m²/sec) for positive and negative responses are 3.089×10^{11} ±7% and 3.105×10^{14} ±7%, respectively. N = 87–103. Arrows indicate controls.

animals probably represent the truer spectral sensitivity curves. Since 500 nm light evoked significant responses during all spectral sensitivity tests, this wavelength was used to determine the phototactic responses of mites to different intensities.

Figure 3 depicts the intensity-dependent phototaxis to 500 nm of light- and dark-adapted animals tested in APW containing no host homogenate. No significant ($P > 0.05$) negative phototaxis occurred in any experiment in which host homogenate was absent from the medium. Dark-adaptation increased the intensity sensitivity of *U. formosa,* resulting in a shift to lower intensities of both maximal phototaxis (from between 1.5×10^{-2} and 1.5×10^{-4} μEinsteins to between 1.5×10^{-3} and 1.5×10^{-6} μE) and the threshold for positive phototaxis (from 1.5×10^{-5} to 1.5×10^{-6} μE). (Note that 1.5×10^{-6} μE of 500 nm light $\cong 3.6 \times 10^{-5}$ μW/cm^2.)

The phototactic sign of animals tested in medium containing host homogenate was a function of intensity (Fig. 4), as the animals were negative to high intensities and positive to low, except for dark-adapted animals at very high intensity (Fig. 4B). The occurrence of negative phototaxis at high intensities (Fig. 4A) implies that the higher the perceived light intensity, the greater the negative response. Thus, it is appropriate to measure the negative response in the determination of spectral sensitivity (Figs. 1, 2). It is unknown why the shift in phototactic sign was less well-defined in dark-adapted animals (Fig. 4B) than in light-adapted mites (Fig. 4A). Dark-adaptation resulted in a shift to lower intensities for maximal negative phototaxis (from 10–10^{-1} μE) and for the point of transition between negative and positive phototaxis (from between 10 and 1 μE to approximately 7.0×10^{-5} μE).

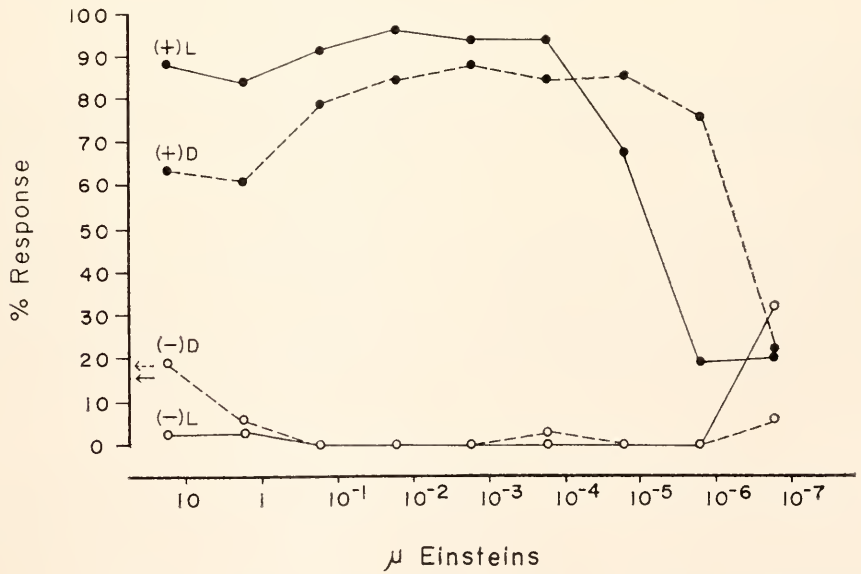

FIGURE 3. Effect of intensity on phototaxis of *U. formosa.* Positive (solid circles) and negative (open circles) phototaxis of both light- (solid lines) and dark-adapted (dashed lines) mites were tested at varying intensities of 500-nm light. All tests were in clean APW. N = 30–36. Arrows indicate controls.

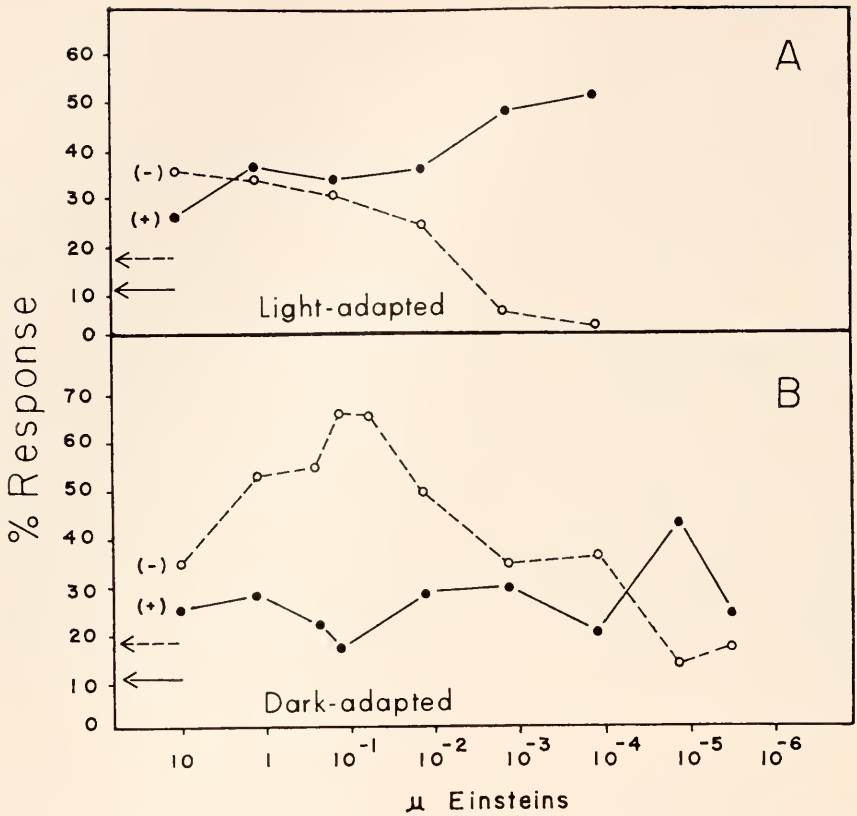

FIGURE 4. Effect of intensity of 500-nm light on phototaxis of light- (A) and dark-adapted (B) mites. Positive (solid line) and negative (dashed line) phototaxis were generated in media containing host homogenate. N = 63 — 68 (A) and 32 — 34 (B). Arrows indicate controls.

Positive and negative phototactic responses of both light- and dark-adapted animals exposed to various wavelengths in APW with host homogenate are depicted in Figure 5. These data are representative of the pattern observed at several quantal intensities. Mites were consistently negatively phototactic at shorter wavelengths but became increasingly positively phototactic at longer wavelengths, a phenomenon particularly evident among light-adapted animals (Fig. 5A).

DISCUSSION

Unionicola formosa exhibited only positive phototaxis when tested in pure APW. In the presence of mantle tissue homogenate from its host, *Anodonta imbecilis,* the sign of the mite's phototaxis was negative to high light intensities. This confirms the work of Welsh (1930).

Maximal sensitivity for positive and negative phototaxis of light-adapted mites occurred at 500 nm. Dark-adapted animals were maximally positively phototactic

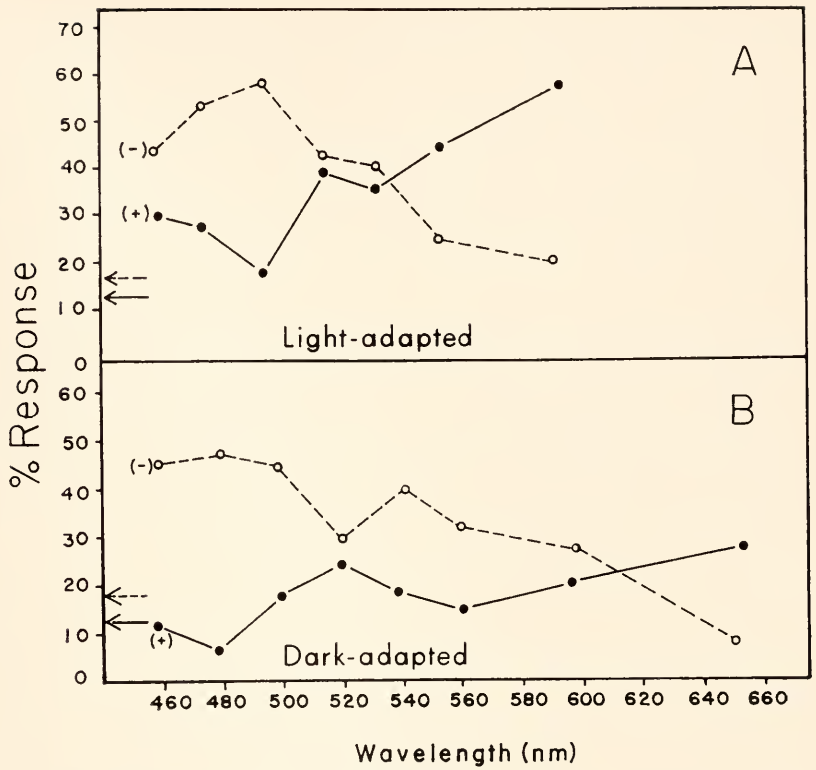

FIGURE 5. Effect of wavelength on the sign of phototaxis of light- (A) and dark-adapted (B) *U. formosa*. Both positive (solid line) and negative (dashed line) phototaxis were generated in test media containing host homogenate. Mean intensity (quanta/m²/sec) is $2.132 \times 10^{18} \pm 16\%$ (A) and $3.105 \times 10^{14} \pm 7\%$ (B). N = 32 — 35 and 92 — 103 for A and B, respectively. Arrows indicate controls.

between 500 to 540 nm, while their negative phototaxis was maximal from 460 to 500 nm. Because dark-adapted animals yield data which are more accurate measures of true sensitivity, it is difficult to define a spectral sensitivity maximum. Waterman (1937), working only with the positive response, found that the velocity of crawling toward a light source by partially light-adapted *U. ypsilophora* (*U. formosa*) was maximal at 430 nm, with lower magnitude responses at 485, 575, and 595 nm. Thus, the spectral sensitivity of *U. formosa* is similar to that of numerous planktonic organisms (Forward, 1976a) and benthic species such as the crayfish *Procambarus clarkii* (Hanaoka, Suganuma, Ikari, and Yasumi, 1957).

Many larval aquatic arthropods are positively phototactic at moderate or low light intensity and photonegative at higher intensities (Thorson, 1964), but positive responsiveness to moderate intensity and avoidance of low intensity light has also been observed (Forward, 1974, 1976b, 1977). No change from positive to negative phototaxis by *U. formosa* occurred in clean APW upon stimulation with the experimental light intensities. However, in the presence of host tissue

homogenate, negative phototaxis was the dominant response to higher intensities while positive phototaxis predominated at lower intensity levels (Fig. 4), a pattern much more pronounced among light-adapted mites (Fig. 4A). The relatively low responsiveness of dark-adapted mites to very high intensities probably was due to overstimulation of the visual system. This is suggested by the observation that dark-adapted mites became disoriented and randomly active in the experimental chamber upon exposure to high intensity stimulation. Dark-adaptation increased the intensity sensitivity of *U. formosa* (Fig. 3), resulting in a threshold for positive phototaxis of about 1.5×10^{-6} μE ($\cong 3.6 \times 10^{-5}$ μ W/cm^2), a value similar to those of other aquatic arthropods (Forward, 1976a).

The patterns of the response spectra of *U. formosa* invite speculation concerning the characteristics of this organism's visual pigment(s). The breadth of the response spectrum for positive phototaxis could result from a single pigment which absorbs strongly over a broad range of wavelengths, a phenomenon not uncommon for invertebrate visual pigments (Wasserman, 1974). Alternatively, multiple pigments could be involved (Waterman, Fernandez, and Goldsmith, 1969). The differences with respect to wavelentgh of the positive and negative phototaxis of *U. formosa* (Fig. 2) suggest the presence of two separate pigments, one absorbing maximally between 460 to 500 nm and responsible for negative phototaxis, the other absorbing maximally in the longer wavelengths and involving positive phototaxis (Fig. 5). Why this change in phototaxis is more pronounced in light- than in dark-adapted animals is unknown. However, the observed differences perhaps can be explained as effects of intensity rather than by invoking the presence of a dual pigment system. The varied response of *U. formosa* tested with 500 nm light in the presence of host homogenate, *i.e.*, negative phototaxis to high and positive to low intensities (Fig. 4), suggests that longer wavelengths may be perceived as of low intensity, to which the more sensitive positive response occurs, while shorter wavelengths may be perceived as of higher intensity, eliciting the negative phototaxis. Thus, "wavelength-dependent" phototaxis may be an intensity effect.

The functional significance of this behavior in *U. formosa* is not completely understood. It has been hypothesized that the chemically mediated reversal of phototaxis serves as a mechanism by which mites remain inside the mantle cavity of a host mussel (Welsh, 1932). Alternatively, negative phototaxis upon exposure to some 'host factor' in the water column could serve to bring specimens of *U. formosa* that are in free-living stages into closer proximity of a benthic potential host mussel. Subsequent analysis may reveal that the observed behavior of adult mites is a retention of larval or nymphal behavior.

Summary

1. The symbiotic mite *Unionicola formosa* is positively phototactic when free of any chemical influence of its molluscan host, *Anodonta imbecilis*, and negatively phototactic in the presence of host tissue homogenate.

2. Light-adapted mites exhibit maximal positive and negative phototaxis at 500 nm, while dark-adapted animals display maximal positive phototaxis between 500 and 540 nm and greatest negative responsiveness between 460 and 500 nm.

3. Dark-adaptation increases the sensitivity of *U. formosa,* resulting in a 1 to 2 log unit shift to lower intensities for maximal phototaxis and of the thresholds for both positive and negative responses.

4. *Unionicola formosa* tested in host homogenate exhibits negative phototaxis at high intensities and postive phototaxis at low intensities.

5. An apparent wavelength-dependent change in phototactic sign is more likely a function of differential sensitivity to intensity.

6. Although presumably a means by which host-symbiont contact is enhanced, the functional significance of the host-influenced behavior of *U. formosa* remains speculative.

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